

Original Article

Biosynthesis of a new psoralidin glucoside by enzymatic glycosylation

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Abstract: Objective To modify the structure of psoralidin using *in vitro* enzymatic glycosylation to improve its water solubility and stability. **Methods** A new psoralidin glucoside (**1**) was obtained by enzymatic glycosylation using a UDP-glycosyltransferase. The chemical structure of compound **1** was elucidated by HR-ESI-MS and nuclear magnetic resonance (NMR) analysis. The high-performance liquid chromatography (HPLC) peaks were integrated and sample solution concentrations were calculated. MTT assay was used to detect the cytotoxicity of the compounds against 3 cancer cell lines *in vitro*. **Results** Based on the spectroscopic data, the new psoralidin glucoside was identified as psoralidin-6',7-di-O- β -D-glucopyranoside (**1**), whose water solubility was 32.6-fold higher than that of the substrate. Analyses of pH and temperature stability demonstrated that compound **1** was more stable than psoralidin at pH 8.8 and at high temperatures. Only psoralidin exhibited a moderate cytotoxicity against 3 human cancer cell lines. **Conclusion** *In vitro* enzymatic glycosylation is a powerful approach for structural modification and improving water solubility and stability of compounds.

Key words: psoralidin; glycosylation; water solubility; stability; cytotoxicity

INTRODUCTION

Psoralidin, 3,9-dihydroxy-2-(3-methylbut-2-enyl)-[1]-benzofuro-[3,2-c]-chromen-6-one, is a natural furano-coumarin isolated from *Psoralea corylifolia* L.^[1] with a wide spectrum of biological activities such as anticancer, antioxidant, antibacterial, antidepressant, and anti-inflammatory activities, and is also shown to regulate insulin signaling^[2-6]. *In vivo* studies of psoralidin, however, are much limited by its poor water solubility and low absorbability^[7]. Glycosylation provides an effective strategy to improve the water solubility, chemical stability, pharmacokinetic properties, and biological potency of many natural products^[8-10]. In this study, we attempted to use *in vitro* enzymatic glycosylation for structural modification of psoralidin to enhance its water solubility and absorbability.

UDP-glycosyltransferase (YjC) is a member of the GT1 family capable of transferring different types of activated sugars (NDP-sugar) to an acceptor. Recent studies have described the use of GTs (YjC) from *Bacillus licheniformis* DSM-13 to synthesize novel glucosides such as mupirocin, apigenin, phloretin, resveratrol, geldanamycin analogs, isobavachalcone, and neolignan^[11-18]. In this study, we report the *in vitro*

glycosylation and examined the structure, water solubility, and stability of the novel psoralidin glucoside.

MATERIALS AND METHODS

Instruments and reagents

Nuclear magnetic resonance (NMR) spectroscopic data were acquired on a Bruker Avance II 600 NMR spectrometer (Bruker, Billerica, MA, USA). HR-ESI-MS spectra were recorded on an Agilent 6538 Accurate Q-TOF mass spectrometer (Agilent Technologies, USA). Semi-preparative reversed-phase high-performance liquid chromatography (HPLC) was carried out on 2535Q (Waters, USA). The YjC enzyme expression vector (pET302-YjC) was obtained from Prof. Jae Kyung Sohng of Sun Moon University. UDP-glucose and other reagents were purchased from Sigma-Aldrich (USA). HPLC-grade acetonitrile and methanol were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Psoralidin was isolated from *P. corylifolia* seeds with a purity exceeding 98%. The structure of psoralidin was confirmed by electrospray ionization-mass spectrometry (ESI-MS) and proton nuclear magnetic resonance spectroscopy (¹H-NMR) of the reference substance^[19].

Enzymatic glycosylation of novel psoralidin glucoside

The expression and purification of YjC was described in detail previously^[15-18]. For *in vitro* glycosylation of

Received: 2016-04-12

Accepted: 2016-05-04

Supported by National Natural Science Foundation of China (81302671) and Grant for Innovative Science Research for Graduates of Bengbu Medical College (Byycx1557).

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psoralidin, a total volume of 50 mL containing 100 mmol/L Tris-HCl (pH 9.6), 1 mmol/L $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 1.5 mmol/L psoralidin, 3 mmol/L UDP-glucose, and 10 mL MeOH was mixed with 35 $\mu\text{g/mL}$ of YjiC. The reaction mixture was incubated at 30 °C for 3 h and quenched twice with an equal volume of EtOAc. The mixture was then centrifuged at 10 000 r/min for 5 min to remove the denatured protein. The EtOAc layer was combined, dried and dissolved in methanol for further analysis and purification. Finally, purification of the product was carried out by semi-preparative HPLC with a SunFire™ C_{18} column (250 mm \times 10 mm, Waters, Milford, MA, USA) connected to a UV detector (254 nm) with 20% acetonitrile ($\text{CH}_3\text{CN}-\text{H}_2\text{O}$, 3.0 mL/min) to yield compound **1** (12 mg).

Water solubility determination

To determine the water solubility of the compounds, an excess of psoralidin and compound **1** were dissolved in 400 μL HPLC-grade water in eppendorf tubes at room temperature. An ultrasonic cleaner was used to maximize the solubility of each compound. After sonication at room temperature for 30 min and centrifugation at 12 000 r/min for 10 min to remove the insoluble material, the solutions were analyzed by HPLC to determine the concentration of the sample solution.

Stability determination

Psoralidin glucoside (**1**) was extracted and purified as described above. To determine the pH value and temperature sensitivity, psoralidin and compound **1** were dissolved in 200 μL Tris-HCl buffer of various pH levels (6.0-9.6) at different temperatures (50-100 °C) for 30 min. The compounds were first dissolved in 200 μL Tris-HCl buffer at pH values of 6.0, 7.0, 8.0, 8.8, and 9.6 for 30 min at room temperature. Similarly, for temperature sensitivity determination, psoralidin and compound **1** were incubated at 50, 60, 70, 80, 90, and 100 °C for 30 min at pH 8.8. Aliquots (20 μL) were used for HPLC analysis to determine the sample solution concentration. The stability of the compounds was calculated as a percentage of the total peak area.

MTT colorimetric assay

Human hepatocellular carcinoma (SMMC7721), breast cancer (MCF-7), and colon adenocarcinoma (SW480) cell lines were grown in DMEM media containing 10% fetal bovine serum (Hyclone, Logan, UT, USA) and 1% penicillin/streptomycin (Gibco, Grand Island, NY, USA). All the cell lines were maintained at 37 °C in a humidified 5% CO_2 incubator. To assess the anti-proliferative activity of the compounds, the cells were seeded in 96-well plates at a density of 6000 cells/well for 1 day, and pre-incubated with the compounds at varying concentrations for 48 h. The anti-proliferative activity of the compounds was evaluated using standard MTT assay procedures^[15, 18].

RESULTS

Identification of psoralidin glucoside

In vitro enzymatic glycosylation was performed and the reaction products were purified by semi-prep HPLC to yield compound **1** (12 mg). Compound **1** was obtained as a white powder, and its molecular formula $\text{C}_{32}\text{H}_{36}\text{O}_{15}$ was established by HR-ESI-MS at m/z 661.2137 $[\text{M} + \text{H}]^+$. The ^1H - and ^{13}C -NMR spectra of compound **1** were similar to those of the substrate, psoralidin^[19]. In contrast, the NMR data of compound **1** showed two anomeric H-atoms δ_{H} 5.04 (^1H , d, $J=7.4$ Hz, H-1''), 5.00 (^1H , d, $J=7.3$ Hz, H-1''') and corresponding C-atoms δ_{C} 100.5 (C-1''), 100.7 (C-1'''), indicating the presence of a disaccharide moiety (Tab.1). The two glucopyranosyl moieties were attached to C-7 and C-6' based on the HMBC correlations from proton at δ_{H} 5.04 (H-1'') to δ_{C} 158.2 (C-7) and δ_{H} 5.00 (H-1''') to δ_{C} 156.7 (C-6'). The HMBC profile also revealed a correlation between the δ_{H} 3.42 (2H, m, H-1'') to δ_{C} 120.8 (C-5), 128.3 (C-6), 158.2 (C-7), showing that the prenyl group was attached to C-6 (Fig.1). In addition, both sugars showed a β -conformation, based on the coupling constant of the anomeric proton at δ_{H} 5.04 ($J=7.4$ Hz) and 5.00 ($J=7.3$ Hz). Therefore, the structure of compound **1** was identified as psoralidin-6',7-di- O - β -D-glucopyranoside (Fig.2).

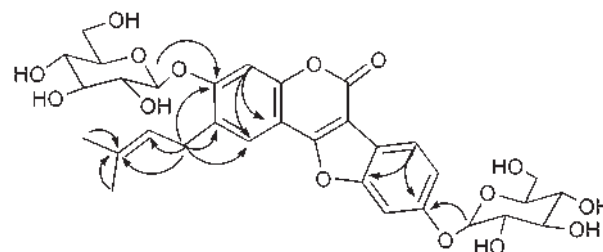


Fig.1 Key HMBC correlations of compound **1**.

Determination of water solubility

The water solubility of the new glucoside (**1**) was evaluated by comparison with that of the substrate. The solubility of compound **1** in water was found to be 527.6 ± 3.42 $\mu\text{mol/L}$, approximately 32.6-fold higher than that of its substrate, psoralidin (16.23 ± 2.31 $\mu\text{mol/L}$). As expected, the enzymatic biosynthesis of a novel glucoside of psoralidin greatly enhanced its water solubility.

Determination of pH and temperature stability

The pH stability and temperature stability of the compounds were determined by incubating psoralidin and its glucoside (**1**) dissolved in 200 μL Tris-HCl buffer at varying pH levels for 30 min at room temperature, and by incubating the compound solutions at different temperatures for 30 min at the most stable pH (8.8). HPLC analysis showed that the novel psoralidin glucoside (**1**) exhibited the highest stability at

Tab.1 ¹H- and ¹³C-NMR (600/150 MHz) data of compound 1 in DMSO-*d*₆

No.	δ_{H}	δ_{C}	No.	δ_{H}	δ_{C}
2	-	157.4	3''	-	132.8
3	-	103.1	4''	1.72 (s)	17.7
4	-	159.7	5''	1.74 (s)	25.6
5	7.73 (s)	120.8	1'''	5.04 (d, 7.4)	100.5
6	-	128.3	2'''	3.26-3.51 (overlap)	76.6
7	-	158.2	3'''	3.26-3.51 (overlap)	73.2
8	7.30 (s)	103.0	4'''	3.26-3.51 (overlap)	77.2
9	-	152.9	5'''	3.18 (m)	63.1
10	-	105.7	6'''	3.73 (m)	60.7
2'	-	155.7	1''''	5.00 (d, 7.3)	100.7
3'	-	116.9	2''''	3.26-3.51 (overlap)	73.3
4'	7.81 (d, 8.5)	120.5	3''''	3.26-3.51 (overlap)	72.5
5'	7.19 (dd, 2.1, 8.5)	115.3	4''''	3.26-3.51 (overlap)	77.1
6'	-	156.7	5''''	3.18 (m)	61.7
7'	7.58 (d, 2.1)		6''''	3.73 (m)	58.4
1''	3.42 (m)	27.6			
2''	5.40 (t, 7.5)	121.6			

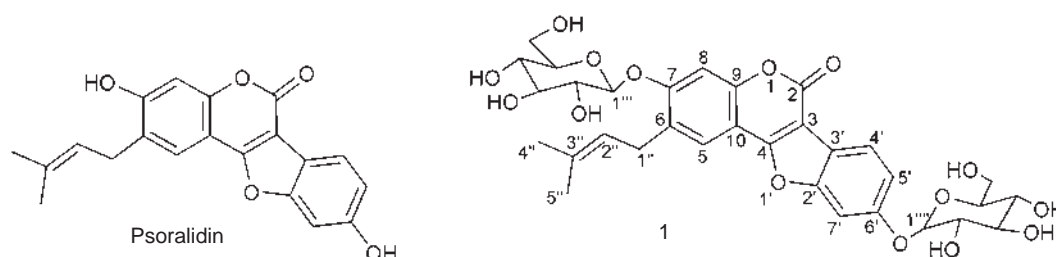


Fig.2 Chemical structures of psoralidin and compound 1.

pH 8.8 with a good temperature stability at 50-70 °C (Fig.3). These results suggested that psoralidin glucoside is relatively stable at pH 8.8 and at high temperatures.

MTT assay for cell viability

We next investigated the anti-proliferative activity of psoralidin and compound 1 against 3 cancer cell lines using MTT colorimetric assay. The results showed that 48 h after addition of the compounds in the cell culture, only psoralidin exhibited a moderate cytotoxicity against the 3 cancer cell lines with IC₅₀ values ranging from 17.46 to 22.62 μmol/L (Tab.2).

DISCUSSION

While natural products provide a rich source of therapeutically useful compounds, the pharmaceutical

industry appears to show reduced interest in the development of natural products^[20, 21]. Moreover, because most natural products display poor physicochemical and pharmacokinetic properties, optimization efforts would preclude semi-synthetic, genetic engineering, biotransformation or efficient route for structure-activity relationship studies.

Glycosylation of secondary metabolites is one of the most common modifications in plants and other produces to confer such physical changes as in water solubility and stability. However, some reactions including glycosylation, which is generally considered to enhance the water solubility, stability, and bioavailability of the substrate, are not easily accessible using chemical methods^[22]. Chemical synthetic approaches for the production of glycosylated compounds involve tedious, time-consuming multi-step chemical reactions. Enzymatic glycosylation method using YjiC offers another option to produce psoralidin

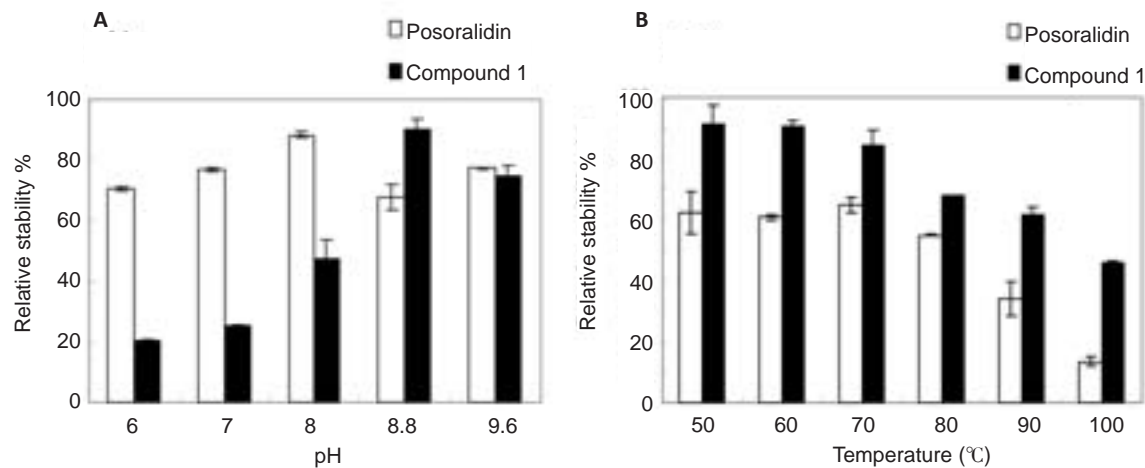


Fig.3 Determination of pH (A) and temperature (B) sensitivities of psoralidin and its glucoside (1).

Tab.2 Anti-proliferative activity (IC₅₀) of the compounds against 3 cancer cell lines (μmol/L, Mean±SD, n=3)

Compounds	SMMC7721	MCF-7	SW480
Psoralidin	20.72±1.33	22.62±1.56	17.46±0.98
Compound 1	>200	>200	>200

glucosides. So far as we know, this is the first report of *in vitro* enzymatic glycosylation of psoralidin using YjiC.

Glycosylation of natural products has emerged as a viable strategy for producing bioactive compounds with an improved activity^[23]. Such sugar attachments also substantially influence pharmacological and pharmacokinetic properties, including tissue specificity, water solubility, distribution, and metabolic stability^[22-24]. The novel psoralidin glucoside we obtained exhibited the highest stability at a pH 8.8 and at 50-70 °C. Therefore, an extraction pH of 8.8 and a temperature around 60 °C can be optimal conditions for biosynthesis of psoralidin glucoside.

To evaluate the pharmaceutical potency, we investigated the anti-proliferative activities of psoralidin and compound 1 against 3 cancer cell lines. Psoralidin is a member of the furanocoumarin subclass of coumarin, and consists of phenolic hydroxyl groups at the C-7 and C-6' positions. C7-OH and C6'-OH form important networks at the active position for biological activities, including antioxidant, antibacterial and anticancer activities^[25]. Thus, the *in vitro* anti-proliferative activity of compound 1 decreased considerably (IC₅₀>200 μmol/L), possibly as a consequence of the bulkiness of glycosylation at the C-7 hydroxyl group and the C-6' hydroxyl group^[15,26].

CONCLUSION

We report the *in vitro* glycosylation of psoralidin by enzymatic biosynthesis. Our data suggest that YjiC is a glycosyltransferase that confers modifications to psoralidin and that glycosylation can improve the water

solubility and stability of compounds. Further studies are needed to clarify whether compound 1 has anti-tumor activity *in vivo*.

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酶法糖基化合成一种新型补骨脂定葡萄糖苷

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摘要:目的 为提高补骨脂定的水溶性和稳定性,用体外酶法糖基化反应对其进行结构修饰。方法 通过UDP-糖基转移酶对补骨脂定进行糖基化修饰,合成一种新的葡萄糖苷化合物(1)。使用高分辨电喷雾电离质谱(HR-ESI-MS)和核磁共振(NMR)分析,鉴定化合物1的结构;利用高效液相色谱峰面积计算出样品溶液的浓度;MTT法检测化合物对3种肿瘤细胞(SMMC7721、MCF-7、SW480)增殖的影响。结果 根据波谱分析,鉴定制备出的新型葡萄糖苷化合物为 psoralidin-6', 7-di-O- β -D-glucopyranoside(1)。水溶性检测结果表明,化合物1的水溶性是底物(补骨脂定)水溶性的32.6倍。此外,化合物1在pH 8.8和高温条件下较补骨脂定更加稳定。在抗肿瘤细胞增殖实验中,只有补骨脂定对3种肿瘤细胞都显示出较强的抑制能力。结论 体外酶法糖基化是进行结构修饰、改善水溶性和稳定性的强有力方法。

关键词:补骨脂定;糖基化;水溶性;稳定性;细胞毒性

收稿日期:2016-04-12

基金项目:国家自然科学基金(81302671);蚌埠医学院研究生科研创新项目(Byycx1557)

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